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Coupling of Semiconductor Nanowires with Neurons and Their Interfacial Structure

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Abstract We report on the compatibility of various nanowires with hippocampal neurons and the structural study of the neuron–nanowire interface. Si, Ge, SiGe, and GaN nanowires are compatible with hippocampal neurons due to their native oxide, but ZnO nanowires are toxic to neuron due to a release of Zn ion. The interfaces of fixed Si nanowire and hippocampal neuron, cross-sectional samples, were prepared by focused ion beam and observed by transmission electron microscopy. The results showed that the processes of neuron were adhered well on the nanowire without cleft.

Keywords Nanowires · Neurons · Coupling · Interfaces · TEM

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Introduction

Semiconductor nanowires have high aspect ratio, high surface area, and single crystallinity and thus are ideal building blocks for many devices on a nanometer scale [1, 2]. Among these, nanowire-based neuron devices that can monitor or stimulate neurons on a submicron dimension with high sensitivity have been recently noticed for their great potential in neuroscience [3]. To realize a nanowire-based neuron device, coupling of nanowires with neurons is essential. Previous studies have shown that the coupling of Si or GaP nanowires with neurons is feasible [4, 5]. However, other semiconductor nanowires that can be considered for neuron devices have not yet been investigated. Meanwhile, monitoring or stimulating of neurons is strongly dependent on the nature of the interfaces between them [6, 7]. For example, the electronic coupling strength between neurons and devices is primarily dependent on the distance between the membrane and the device surface [8, 9]. In fact, the weak coupling between neuron and devices due to the extracellular cleft is one of the major problems in neuron-electronic interfaces. Analysis of the interfacial structures is thus essential in the design of nanowire-based neuron devices as well as for understanding the signal transfer mechanism. In the present study, we investigate the coupling of group IV (Si, Ge and SiGe), III-V (GaN), and oxide (ZnO) semiconductor nanowires with hippocampal neurons that are believed to be involved in the general and spatial memory, and characterize the coupled interface via transmission electron microscopy (TEM). Our results indicate that IV and III-V semiconductor nanowires are compatible with the neurons, whereas oxide semiconductor nanowires are not compatible. Characterization of the coupled Si nanowire–neuron interfaces shows two layers comprised of a coupling modifier and natural oxides

with a thickness of ~8 nm. No clefts were found at the interfaces.

Experimental Procedure

Synthesis of Nanowires

We synthesized Si (a), SiGe (b), Ge (c), and GaN (d) nanowires on a (a–c) Si (111) and (d) c-plane sapphire substrates coated with (a–c) Au, and (d) Ni as a VLS catalyst by a conventional CVD process employing (a) silicon tetrachloride ($SiCl_4$, Alfa, 99.999%) as a silicon source, (b) $SiCl_4$ as a silicon source and germanium powder as the germanium source, (c) germanium tetrachloride ($GeCl_4$, Alfa, 99.999%) as a germanium source, and (d) metallic Ga powder as a gallium source and ammonium gas as a nitrogen source [10–13]. The substrates were placed in the center of quartz tube, and powder sources were also placed at the near of substrates with a distance of 1 in. Carrier gas transfers the source precursor through a bubbler to the quartz reactor, and hydrogen and argon gas were used as diluent gases, which regulate the concentration of the mixture containing source gas and carrier gas. The temperature of the furnace was increased at a heating rate of 50 °C min^{-1} to 800 °C under flow of source and carrier gases and kept for 10 through 60 min and then cooled down to room temperature. ZnO nanowires were grown by a typical carbothermal reduction process. An equal amount of ZnO and graphite powders were mixed and transferred to an alumina boat inside the processing tube. The processing temperature varied from 800 to 950 °C [14]. The all prepared nanowires were observed by a scanning electron microscopy.

Cell Culture

The nanowires were dispersed in ethanol and laid on the Si wafer. After sterilization by ethanol and UV light, the surface of the nanowires was chemically modified by a poly-L-lysine (PLL) coating for cell adhesion. Hippocampal neurons were then cultured on the nanowires. Briefly, the hippocampal neurons were isolated from 16- to 18-day-old fetal Sprague–Dawley rats and incubated with 0.25% trypsin Hanks balanced salt solution (HBSS) at 37 °C for 15 min. Cells were then mechanically dissociated with fire-polished Pasteur pipettes by trituration and plated on prepared substrata in a 24-well-plate culture dish. Cells were maintained in Neurobasal/B27 medium containing 0.5 mM L-glutamine, 25 μM glutamate, 25 μM 2-mercaptoethanol, 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. 50,000 cells were incubated with a substrate deposited in a well of the 24-well plate at 37 °C in 5% CO_2 incubator.

Results and Discussion

Figure 1a–e are a typical SEM images of various NWs grown on the substrate. The NWs grew with the diameter

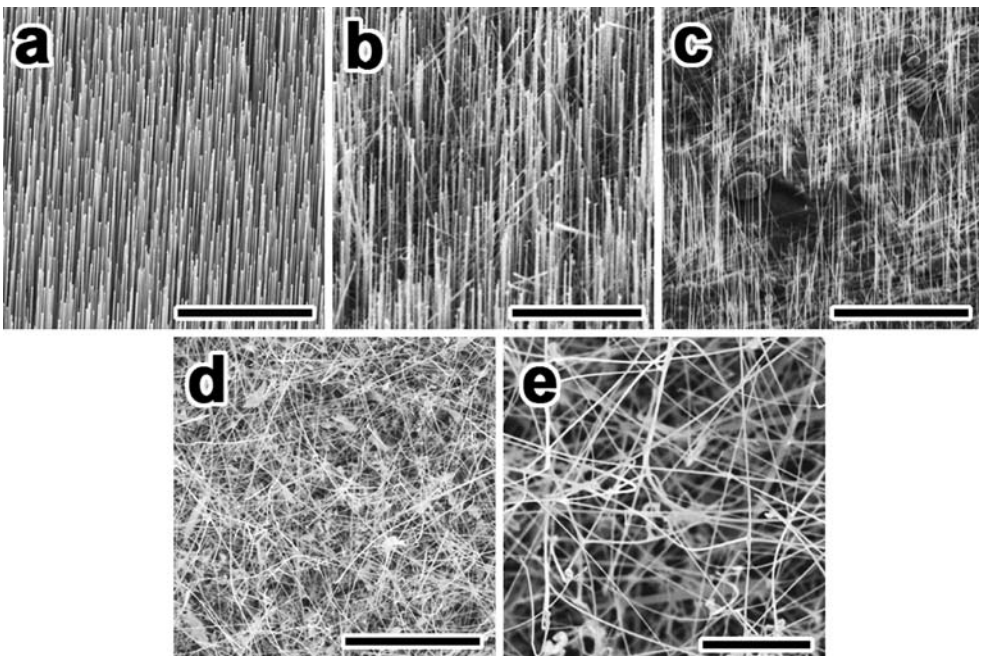

Fig. 1 Typical SEM image of Si (**a**), SiGe (**b**), Ge (**c**), GaN (**d**) and ZnO (**e**) nanowires. *Scale bar* is 60 μm in **a**, 30 μm in **b**, 10 μm in **c** and **d**, and 3 μm in **e**

ranging from 40 to 150 nm and lengths of several tens micrometer, respectively.

Figure 2a–f show SEM images of hippocampal neurons cultured for 4–5 days on the nanowires. To observe the morphology of the neuron cells by scanning electron microscopy (SEM), they were treated via a critical point drying technique after the treatment with glutaraldehyde for fixation and osmium tetroxide for contrast enhancement. In comparison with a standard sample (i.e., cultured neurons on a PLL-coated silica substrate), hippocampal neurons were grown similar to a standard one with many protruding processes, except for the case of ZnO nanowires. This outcome implies that, in addition to Si nanowires [4], which have already shown a compatibility with neurons, SiGe, Ge, and GaN nanowires are compatible with hippocampal neurons. Our previous studies have shown that the surfaces of Si, SiGe, Ge, and GaN nanowires consist of SiO_x, SiO_x, GeO_x, and Ga_xO_y, respectively, [11, 15–17] as a result of natural oxidation. These native oxides come into substantial contact with the neurons and may contribute to the compatibility of the nanowires. SEM images of the neurons cultured with ZnO nanowires, meanwhile, reveal that growth processes were less developed in comparison with the other cases. This indicates that these nanowires may be toxic to neurons. To verify this, a MTT assay, a technique widely used to measure cell viability, was performed on the neurons cultured with ZnO nanowires, and the results were compared with those obtained for Si nanowires. Si and ZnO nanowires were incubated with hippocampal neuron (50,000 cells). For adhesion cells, we removed the media and replace it with fresh culture medium. For labeling, we added 12-mM MTT stock (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated at 37 °C for 3–4 h. For detecting, we added dimethyl sulfoxide (DMSO) to each well and mix thoroughly with the pipette and incubated at 37 °C for 30 min with shaking helps solvate well the formazan. We read absorbance at 540 nm. Earlier mentioned procedure was performed at 0, 24, 48, and 72 h. As shown in Fig. 3, the activity of neurons cultured with ZnO nanowires decreased with culture time while that with Si nanowires increased. This shows that ZnO nanowires are

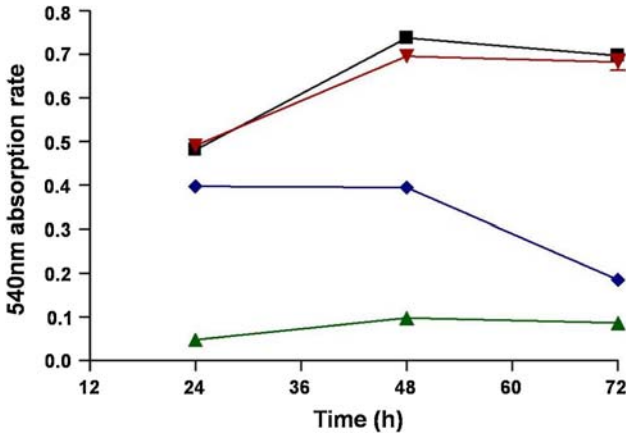

Fig. 3 The absorption rate result of MTT assay for hippocampal neuron in 72 h (*filled square*: control, *filled triangle*: negative control, *filled down pointing triangle*: Si NW, *filled diamond*: ZnO NW, at 540 nm)

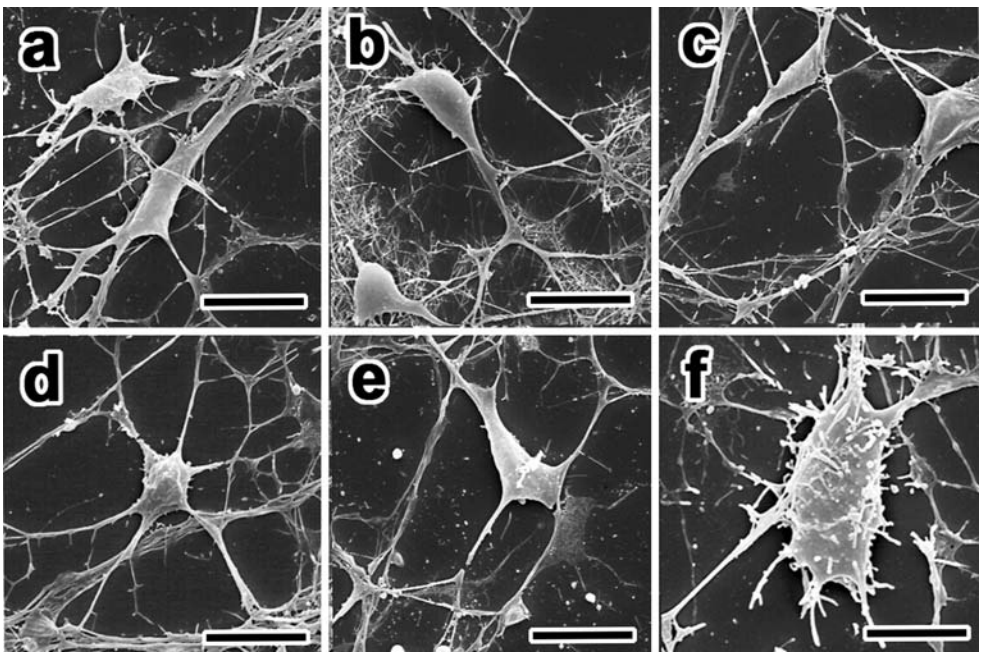

Fig. 2 SEM images of 4–5 days cultured hippocampal neurons with various nanowires (**a**: PLL-coated silica substrate without nanowires, **b**: Si, **c**: SiGe, **d**: Ge, **e**: GaN, **f**: ZnO, *Scale bar* is 15 μm in **a**–**e** and 6 μm in **f**)

toxic to hippocampal neurons. One possible explanation for this is dissolution of Zn from the nanowires in the course of culturing. It is known that Zn release contributes to neural death [18, 19]. To confirm this, the culture media with ZnO and Si nanowires were analyzed by inductively coupled plasma mass spectroscopy (ICP). The results showed revealed Zn content of 6.6 ppm and Si content of <2 ppm, where the former is ten times higher than the value of the standard sample and the latter corresponds with those, respectively. Therefore, it could be concluded that the dissolution and release of Zn ions are responsible for the neurotoxicity of the ZnO nanowires.

Since Si nanowires were identified as being biocompatible to neurons in the SEM, MTT assay, and ICP analyses, the Si nanowire–neuron couple was selected to investigate the interfacial structure. We prepared ultrathin cross-sectioned samples and characterized by using TEM for direct observation of interfaces on a nanometer scale. The couples were first dried by critical point drying technique that is widely used to observe cellular morphology without deformation [20, 21]. After drying treatment, the coupled interface was cross-sectioned using a high-resolution Cross Beam FIB-FESEM instrument, and the sidewall of the cross section was polished with a low-ion current and imaged in situ by SEM until a width of less than 80 nm, after which it was observed by TEM. Figure 4a shows the one of the coupled neurons with Si nanowires where the neuron wraps the nanowires in an omega (Ω) shape. Figure 4b shows a cross-sectioned image of the neuron–nanowire interface. The entire cross-sectional interfacial structure was well preserved, and distinct shrinking artifacts were not found. Figure 4c–e show the

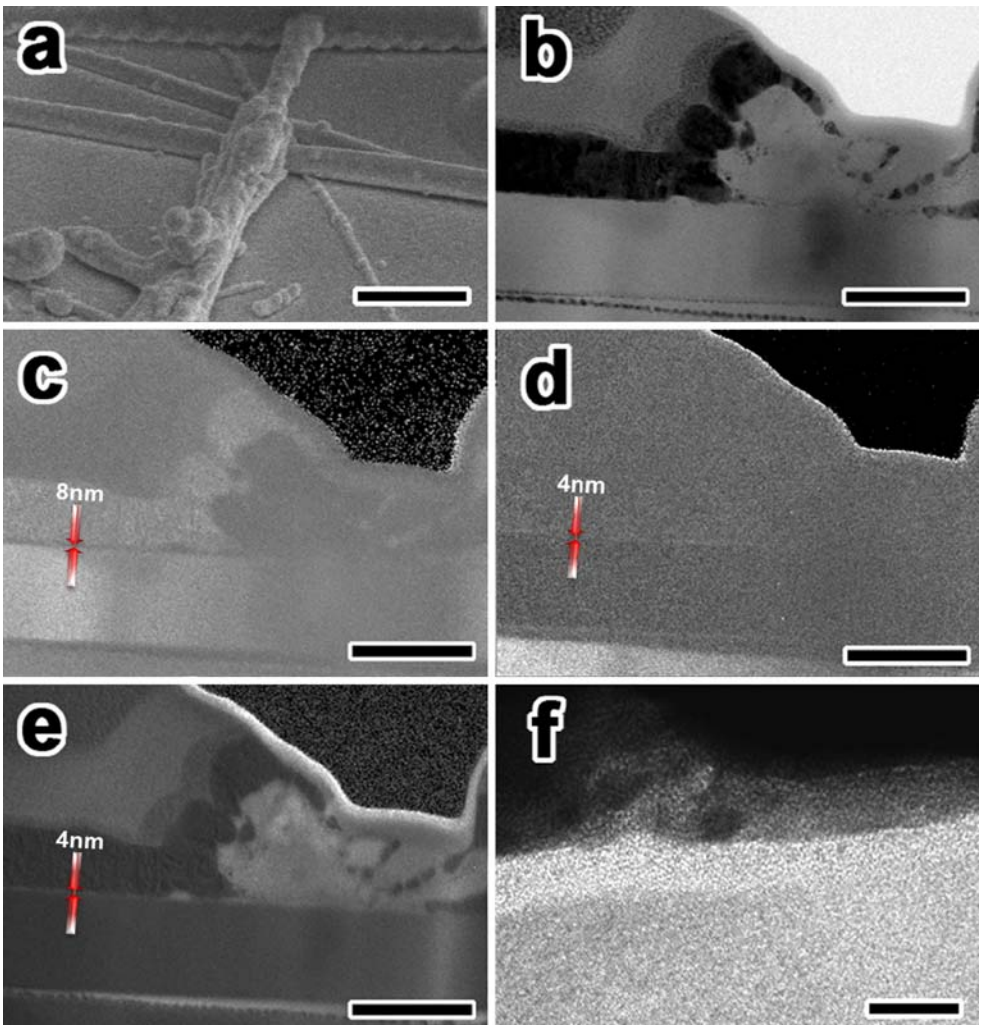

Fig. 4 **a** SEM image of coupled Si nanowire with neuronal process. **b** Cross-sectioned image of neuron–nanowire interface showing neuron (N), Si nanowires (Si), gold (Au) and platinum (Pt) films deposited for focused ion beam process. **c–e** Element mapping of cross-sectional interfaces obtained by jump-ratio method in TEM analysis (**c**: silicon, **d**: oxygen, **e**: carbon). Each mapping shows ~8, ~4, and ~8 nm of interfacial layers, respectively, **f** High-resolution TEM image of interface showing ~4 nm of SiO_2 (*bright*, *below*) and ~4 nm of PLL (*dark*, *above*) layers. *Scale bar* is 1 μm in **a**, 200 nm in **b–e** and 10 nm in **f**

representative results of element mapping of cross-sectional interfaces obtained by the jump-ratio method in the TEM analysis. The silicon jump-ratio image shows the Si nanowire (Fig. 4c), the oxygen jump-ratio image shows the silicon oxide layer (Fig. 4d), and the carbon jump-ratio image shows the PLL layer and neuronal process with bright contrast (Fig. 4e), respectively. The analysis revealed that the neuronal process attached tightly to the Si nanowire without any cleft, and the interfaces consisted of a multilayer of neuron/PLL/SiO_2/nanowires. The high-resolution TEM image (Fig. 4f) also shows an interfacial layer with a thickness of about 8 nm consisting of a ~4-nm layer of SiO_2 and ~4-nm PLL layer.

In the earlier mentioned characterization studies, no clefts, which might be caused by filled culture medium before drying, were found. In the previous characterizations of the interfaces between human embryonic kidney (HEK) cell and a Si field effect transistor (FET) [21] or cells on a SiO_2 substrate [22], cleft with an average width of roughly 40 nm was observed, depending on the type of modifier. It is not clear why such clefts have not been observed in the present neuron–nanowires interfaces. It may due to the different growth behavior of the neurons on the nanostructured surfaces formed by the nanowires when compared to the flat FET surface [23] or the small contact area on a nanometer scale. Regardless of the mechanism, the neuron–nanowire couples may be advantageous for the development of neuron devices in terms of signal transfer and electronic coupling, since the clefts pose critical problems in relation to signal transfer and electronic coupling strength.

Many approaches can be considered for the fabrication of nanowire-based neuron devices, including coupling nanowire transistors to neurons [24, 25] and probing neurons with vertical nanowire array [26]. In all of these cases, the signal is transferred through the interface. In this regard, the formation of tight-, very thin interfaces between nanowires and neurons would lend promise for monitoring and/or stimulating of neurons. Furthermore, as shown in Fig. 4a, the neurons can wrap the nanowires in a Ω shape or totally in the case of a vertical array. This aspect also is potentially advantageous for highly sensitive monitoring and/or stimulating of neurons, since and omega- or all-surround gating effect is expected, akin to advanced transistor structures [27].

Conclusion

In summary, we investigated the compatibility of various nanowires with hippocampal neurons. Si, Ge, SiGe, and GaN nanowires were found to be compatible to neurons under the present culturing conditions. However, ZnO nanowires are toxic to neurons as a result of the release of Zn ions from the nanowires. The interface of coupled Si nanowires and neurons shows no clefts and is comprised of a SiO_2 layer of 4 nm and a PLL coating layer of 4 nm. Formation of omega-shaped, tightly bonded interfaces with very thin interfacial layers is promising for monitoring and/or stimulating neurons by nanowires.

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